## REMARKS

### I. Amendments to the Claims

Claims 4, 8, 11, 15, 19, 21, 22, and 24 are amended herein solely to address formalities and correct errors of an inadvertent or typographic nature. The amendments to claims 8, 11, 19, and 22 find support in the Specification in at least page 4, lines 21 to 32. Claims 72-75 are newly added. The added claims find support in the Specification in original claims 4, 11, 15 and 22; and on at least page 3, line 32 to page 5, line 28; page 27, lines 20 to 30; and page 30, lines 11 to 31. No new matter is added by these amendments.

Claims 23 to 71 are withdrawn, pursuant to a requirement for restriction. Applicants reserve the right to pursue the withdrawn claims in one or more divisional applications.

Applicants respectfully request consideration and allowance of the pending claims.

### II. Objection to the Specification

The Examiner has objected to the Specification for not capitalizing terms containing alleged trademarks, such as ROSETTA, TUNER, and ORIGAMI. See Office Action, page 3. Applicants agree that ROSETTA, TUNER and ORIGAMI are trademarks used to refer to groups of bacterial strains, however, the instant specification does not refer to the groups, but only refers to the individual strains, e.g. "Origami(DE3)."

A search of the USPTO Trademark Electronic Search System for each of the terms ROSETTA, TUNER, and ORIGAMI did not identify any individual strain identifiers that are trademarks. Furthermore, the owner of the aforementioned trademarks, EMD Biosciences, Inc., refers to each of the strains recited in the Specification without any indication that the names of the individual strains are trademarks, and includes ROSETTA, TUNER, and ORIGAMI in its list of trademarks but omits each of the

individual strain identifiers. See Novagen 2004/2005 Catalog, pages 116-117 and 406 (Appendix A). Note that Novagen is a subsidiary of EMD Biosciences, Inc.

Thus, the names of the individual strains referenced in the instant specification do not appear to be trademarks. Nonetheless, solely to advance the prosecution of this case, Applicants have amended the Specification to capitalize each of these terms where they appear as part of a strain identifier, rendering this basis for objection moot. Accordingly, withdrawal of the objection is respectfully requested.

## III. Objection to the Claims

The Examiner objects to claims 5 and 16 as being of improper dependent form for allegedly "failing to further limit the subject matter of a previous claim under 35 USC § 112, ¶ 4." See Office Action, page 3. The MPEP sets forth the standard for determining compliance with 35 U.S.C. § 112, fourth paragraph, as follows:

> A dependent claim does not lack compliance with 35 U.S.C. 112, fourth paragraph, simply because there is a question as to (1) the significance of the further limitation added by the dependent claim, or (2) whether the further limitation in fact changes the scope of the dependent claim from that of the claim from which it depends. The test for a proper dependent claim under the fourth paragraph of 35 U.S.C. 112 is whether the dependent claim includes every limitation of the claim from which it depends. The test is not one of whether the claims differ in scope.

See MPEP 8th ed. rev. 6, § 608.01(n).

Thus, the relevant inquiry for determining compliance with 35 U.S.C. § 112, fourth paragraph is whether a dependent claim includes every limitation of the claim from which it depends, not whether the claims differ in scope. Claims 5 and 16 each incorporate by reference the limitations of the claims from which they depend and further recite: "wherein the mature SpeB polypeptide is immunogenic in a mammalian host." That further recitation does not vitiate the limitations of the claims from which claims 5 and 16 depend. As claims 5 and 16 include every limitation of the claims from

which they depend, they are in proper dependent form. Accordingly, Applicants respectfully request reconsideration and withdrawal the rejection.

## IV. Reply to Rejections Under 35 U.S.C. § 101

The Examiner rejects claims 1-8 and 12-19 as allegedly encompassing non-statutory subject matter, specifically a human. Applicants respectfully traverse.

The cited basis for the rejection, based on the standard promulgated in 1077 O.G. 24, April 21, 1987, is set forth in the MPEP as follows:

> If the broadest reasonable interpretation of the claimed invention as a whole encompasses a human being, then a rejection under 35 U.S.C. 101 must be made indicating that the claimed invention is directed to nonstatutory subject matter.

See MPEP 8th ed. rev. 6 § 2105. Thus, this basis for rejecting claims applies to *compositions* that could be construed to encompass a human. It does not apply to methods, including methods involving human cells.

Claims 1-8 and 12-19 are *methods* for recombinantly expressing a mature SpeB polypeptide in a host cell. Whether the host cell is a human is irrelevant because claims 1-8 and 12-19 do not claim the host cell, and thus do not encompass a composition comprising a human. Accordingly, Applicants respectfully request reconsideration and withdrawal the rejection.

## V. Reply to Rejections Under 35 U.S.C. § 112, First Paragraph

### A. Claims 6 and 17 satisfy the written description requirement.

The Examiner has rejected claims 6 and 17 as allegedly failing to comply with the written description requirement for including the phrase "wherein an antibody specific for the mature SpeB polypeptide cross-reacts with a wild-type SpeB polypeptide and neutralizes SpeB polypeptide activity." The Examiner alleges that:

> To adequately describe the genus of methods for expressing a mature SpeB polypeptide, wherein the mature SpeB polypeptide binds and is neutralized by antibodies that are cross-reactive with wild-type SpeB

> polypeptide, applicant must adequately describe the antigenic determinants (immunoepitopes) that elicit the required cross-reactive and neutralizing antibodies directed against SpeB polypeptides.

See Office Action, page 4-5.

Applicants respectfully request reconsideration and withdrawal of the rejection because (1) the Specification discloses actual reduction to practice of the claimed invention; (2) identification of immunoepitopes is not required to claim antibodies to an antigen; and (3) the Specification discloses a particular sequence and an assay for functionality.

**1. The Specification describes an actual reduction to practice of the claimed invention to satisfy the written description requirement.**

As correctly recognized by the Examiner, the written description requirement of 35 U.S.C. § 112, paragraph 1, can be met "in a variety of ways including *description of an actual reduction to practice*... to show that the Applicants were in possession of the claimed invention." See Office Action, page 6.

Claims 6 to 17 are directed to methods "wherein an antibody specific for the mature SpeB polypeptide cross-reacts with a wild-type SpeB polypeptide and neutralizes SpeB polypeptide activity." Example 6 (Figure 11) of the instant Specification shows an actual reduction to practice of the claimed invention by showing antisera raised against mature SpeB cross-reacting with a wild-type SpeB polypeptide and neutralizing SpeB polypeptide activity. The example demonstrates similar results obtained with SpeB obtained using both a polycistronic system (an embodiment of claim 6) and a two-plasmid system (an embodiment of claim 17). As the Examiner has correctly noted, an actual reduction to practice satisfies the written description requirement. See *Enzo Biochem Inc. v. Gen-Probe, Inc.*, 323 F.3d 956, 964 (Fed. Cir. 2002). Accordingly, Applicants respectfully request reconsideration and withdrawal the rejection.

**2. Identification of immunoepitopes is not required to claim antibodies to an antigen**

Greenspan *et al.* explores "the critical assumptions of alanine scanning mutagenesis" and compares "the different aspects [of the antibody-antigen interaction] that are captured by structural versus mutational methods." See Greenspan *et al.*, abstract. The Examiner alleges that "[a]s evidenced by Greenspan *et al.* (Nature Biotechnol. 7:936-937, 1999) defining epitopes is not as easy as it seems."

Greenspan *et al.* is not applicable to the instant case because Applicants need not define immunoepitopes to claim methods of using antibodies that interact with SpeB. The Federal Circuit has held that:

> disclosure of an antigen fully characterized by its structure, formula, chemical name, physical properties, or deposit in a public depository provides an adequate written description of an antibody claimed by its binding affinity to that antigen.

See MPEP 8th ed. rev. 6 § 2163 (citing Noelle v. Lederman, 355 F.3d 1343, 1349, 69 USPQ2d 1508, 1514 (Fed. Cir. 2004)). Thus, *identification of immunoepitopes is not required to provide adequate written description of antibodies claimed by their binding affinity to an antigen.*

Here, the Specification discloses an antigen, mature SpeB polypeptide. Under the standard promulgated in *Noelle v. Lederman*, this disclosure provides sufficient written description support for claims directed to antibodies reactive against that SpeB polypeptide. Accordingly, Applicants respectfully request reconsideration and withdrawal the rejection.

**3. The Specification discloses a particular sequence and an assay for functionality**

The present case is analogous to Example 14 presented in the Synopsis of Application of Written Description Guidelines ("Synopsis"), which provides useful guidance in determining the allowable claim scope with respect to the written

description requirement. See http://www.uspto.gov/web/menu/written.pdf. Example 14 of the Synopsis considers the following hypothetical claim:

> A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A → B.

The example explains that the procedures for making variants (*e.g.*, substitutions, deletions, insertions, and additions) of the recited sequence are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Further, procedures for making variants of the recited sequence which have 95% identity to the recited sequence and retain its activity are conventional in the art. In addition, there is an actual reduction to practice of the recited sequence, and the Specification indicates that the genus of proteins that must be variants of the recited sequence does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the recited sequence. Specifically, the PTO concludes that the hypothetical claim provides adequate written description for the following reasons:

> There is actual reduction to practice of the single disclosed species [SEQ ID NO: 3]. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation *since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3.* The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the *presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity.* One of skill in the art would conclude that applicant was in possession of the necessary common attributes possessed by the members of the genus.

Synopsis, page 54 [emphasis added].

Example 14 teaches that when an application describes (1) a particular sequence and (2) an assay for determining functionality, the application contains sufficient written description for claims directed to the sequence itself, as well as variants thereof that have a degree of homology and that maintain functionality. See Synopsis, Example 14.

As in Synopsis Example 14, Applicants have disclosed a sequence (mature SpeB polypeptide), and an assay for determining functionality of that sequence (the assay described in Example 6 of the instant Specification). As in Example 14 one of skill in the art would thus conclude that Applicants were in possession of the necessary common attributes possessed by the members of the genus. Accordingly, Applicants respectfully request reconsideration and withdrawal the rejection.

**B. Claims 8 and 19 are enabled.**

The Examiner has rejected claims 8 and 19 as allegedly containing subject matter which was not described in the Specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the Examiner alleges that the plasmid pCRT7-CTTOPO is required to practice the invention and that this allegedly necessitates the deposit of biological material by the Applicants. Applicants respectfully traverse.

A deposit of biological materials is not required because pCRT7-CTTOPO could be made without undue experimentation.

**pCRT7-CTTOPO could be made without undue experimentation.**

A deposit of biological material is not required when that material can be made or isolated without undue experimentation:

> Applicant may show that a deposit is not necessary even though specific biological materials are required to practice the invention if those biological materials can be made or isolated without undue experimentation. Deposits may be required to support the claims if an isolation procedure requires undue experimentation to obtain the desired biological

> material. Ex Parte Jackson, 217 USPQ 804 (Bd. App. 1982). No deposit is required, however, where the required biological materials can be obtained from publicly available material with only routine experimentation and a reliable screening test. Tabuchi v. Nubel, 559 F.2d 1183, 194 USPQ 521 (CCPA 1977); Ex Parte Hata, 6 USPQ2d 1652 (Bd. Pat. App. & Int. 1987).

See MPEP 8th ed., rev 6, § 2404.02. The sequence of pCRT7-CTTOPO is published. See http://www.invitrogen.com/content/sfs/vectors/pcrt7cttopo_seq.txt. As the sequence is a mere 2.7 kb in length, it could be made by persons skilled in the art without undue experimentation, using well known synthesis procedures. For example, a 5.4 kb bacteriophage genome was created using synthetic oligonucleotides in less than two weeks. See Smith *et al.* "Generating a synthetic genome by whole genome assembly: {phi}X174 bacteriophage from synthetic oligonucleotides". Proceedings of the National Academy of Sciences 100 (26): 15440-15445, 2003. The same procedure could readily be used to produce a 2.7 kb plasmid sequence. Applicants further note that pCRT7-CTTOPO is widely used by the scientific community, as evidenced by its use in over two hundred recent scientific publications. See http://scholar.google.com/scholar?q=pCRT7.CT.TOPO+OR+pCR.T7.CT.TOPO. The plasmid is also commercially available from INVITROGEN. See http://www.invitrogen.com/content.cfm?pageid=8012&sku=K421001.

As pCRT7-CTTopo can be made or isolated without undue experimentation, no deposit of biological material is required. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

## VI. Reply to Rejections Under 35 U.S.C. § 112, Second Paragraph

### A. Claims 4 and 15 are definite.

The Examiner has rejected claims 4 and 15 as allegedly vague and indefinite. Specifically, the Examiner alleges that the phrase "wherein the cysteine at amino acid residue 192 of the mature SpeB polypeptide is substituted by serine" is vague and indefinite because multiple sequences for this protein are known in the art. See Office

Action, page 8. Applicants believe that a person skilled in the art would understand the metes and bounds of the invention based on Applicants' disclosure; however, solely to advance the prosecution of this case, the claims are amended to recite:

> 4. The method of claim 1, wherein the mature SpeB polypeptide is further defined as a polypeptide comprising amino acid residues 146 through 398 of SEQ ID NO:2 wherein the cysteine at amino acid residue 192 ~~of the mature SpeB polypeptide~~ is substituted by a serine.
>
> 15. The method of claim 12, wherein the mature SpeB polypeptide is further defined as a polypeptide comprising amino acid residues 146 through 398 of SEQ ID NO:2 wherein the cysteine at amino acid residue 192 ~~of the mature SpeB polypeptide~~ is substituted by a serine.

As the basis for the rejection is rendered moot by the amendment, Applicants respectfully request withdrawal of the rejection.

**B. Claims 6 and 17 are definite.**

Applicants note an apparent typographical error in the Office Action, namely that claim 19 is indicated in this rejection where the quoted text clearly indicates that claim 17 was intended. Applicants respond accordingly, and request clarification if this interpretation is incorrect.

The Examiner has rejected claims 6 and 17 as allegedly vague and indefinite. Specifically, the Examiner alleges that it is not clear whether the phrase "wherein an antibody specific for the mature SpeB polypeptide cross-reacts with a wild-type SpeB polypeptide and neutralizes SpeB polypeptide activity" refers to additional method steps or whether this phrase is merely describing a property of the mature SpeB polypeptide. Applicants respectfully traverse.

"The words of a claim must be given their 'plain meaning' unless such meaning is inconsistent with the specification." See MPEP 8th ed., rev. 6, § 2111.01. The term "wherein" has the plain meaning "in which," and is traditionally used in claim language to define properties of a claim element. The Examiner has not identified any definition or

usage of the term "wherein" in the Specification that is inconsistent with plain meaning, and thus the term "wherein" carries its plain meaning and is definite. Therefore, it is clear that the phrase "wherein an antibody specific for the mature SpeB polypeptide cross-reacts with a wild-type SpeB polypeptide and neutralizes SpeB polypeptide activity" describes a property of the mature SpeB polypeptide. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

**C. Claims 11 and 22 are definite.**

The Examiner has rejected claims 11 and 22 as allegedly using a trademark or trade name in a claim as a limitation to identify or describe a particular material or product, specifically for recitation of the strain identifiers: BLR(DE3), BLR(DE3)pLysS, AD494(DE3), AD494(DE3)pLysS, BL21(DE3), BL21(DE3) pLysS, BL21(DE3)pLysE, BL21(DE3)pLacI, BL21trxB(DE3), BL21trxB(DE3)pLysS, HMS174(DE3), HMS174(DE3)pLysS, HMS174(DE3)pLysE, Origami(DE3), Origami(DE3)pLysS, Origami(DE3)pLysE, Origami(DE3)pLacI, OrigamiB(DE3), OrigamiB(DE3)pLysS, OrigamiB(DE3)pLysE, OrigamiB(DE3)pLacI, Rosetta(DE3), Rosetta(DE3)pLysS, Rosetta(DE3)pLysE, Rosetta(DE3)pLacI, Tuner(DE3), Tuner(DE3)pLysS, and Tuner(DE3)pLacI. Applicants respectfully traverse.

As discussed (see "Objection To The Specification," above) the strain identifiers recited in the claims are *not trademarks*. The terms ROSETTA, TUNER, and ORIGAMI are trademarks that refer to groups of strains, but the individual strain identifiers are not trademarks. Rather, they are "names used in trade" which are expressly permitted in patent claims:

> *Names Used in Trade*: a nonproprietary name by which an article or product is known and called among traders or workers in the art, although it may not be so known by the public, generally. Names used in trade do not point to the product of one producer, but they identify a single article or product irrespective of producer. Names used in trade are permissible in patent applications if:

> (A) Their meanings are established by an accompanying definition which is sufficiently precise and definite to be made a part of a claim, or
>
> (B) In this country, their meanings are well-known and satisfactorily defined in the literature.

See MPEP 8th ed., rev. 6, § 608.01(v).

The lineage and genetic characteristics of each strain are fully defined in the literature, e.g. in catalogs readily available in this country offering these strains for sale. See Novagen 2004/2005 Catalog, pages 114-115 (Appendix A). The defined lineage and genetic characteristics identify a single article or product irrespective of producer, thus the strain identifiers are names used in trade. Claims are expressly permitted to include names used in trade, and are not rendered indefinite by their usage. See MPEP 8th ed., rev. 6, § 608.01(v). Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

**D. Claims 8 and 19**

The Examiner has rejected claims 8 and 19 as allegedly vague and indefinite for the use of the terms "pET, pRSET, pCRT7-CTTOPO, and pIVeX." Specifically, the Examiner alleges that these terms are laboratory designations that do not provide any structural or functional limitations. Applicants respectfully traverse.

A "laboratory designation" is a nonproprietary name by which an article or product is known and called among practitioners of laboratory science. Thus, a "laboratory designation" is a "name used in trade." As noted above, "names used in trade" are expressly permitted if "[i]n this country, their meanings are well-known and satisfactorily defined in the literature." See MPEP 8th ed., rev. 6, § 608.01(v).

The DNA sequence of each vector is published and therefore "their meanings are well-known and satisfactorily defined in the literature." The use of such satisfactorily defined names used in trade in patent applications is expressly permitted. *Id.* Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

## VII. Reply to Rejections Under 35 U.S.C. § 103(a)

### A. Claims 12-22 are not obvious

The Examiner has rejected claims 12-22 as allegedly obvious over Gubba *et al.* (Infect. Immun., 66:765-770, 1998) in view of Matsuka *et al.* (Infect. Immun., 67:4326-4333, 1999).

The Examiner alleges that "it is obvious to combine prior art elements according to known methods to yield predictable results. The MPEP sets forth the requirements to support such a rejection:

> Combining Prior Art Elements According to Known Methods To Yield Predictable Results
>
> To reject a claim based on this rationale, Office personnel must resolve the Graham factual inquiries. Then, Office personnel must articulate the following:
>
> (1) a finding that the prior art included each element claimed, although not necessarily in a single prior art reference, with the only difference between the claimed invention and the prior art being the lack of actual combination of the elements in a single prior art reference;
>
> (2) a finding that one of ordinary skill in the art could have combined the elements as claimed by known methods, and that in combination, each element merely performs the same function as it does separately;
>
> (3) a finding that one of ordinary skill in the art would have recognized that the results of the combination were predictable; and
>
> (4) whatever additional findings based on the Graham factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.
>
> ....
>
> If any of these findings cannot be made, then this rationale cannot be used to support a conclusion that the claim would have been obvious to one of ordinary skill in the art.

See MPEP 8th ed. rev 6, § 2143, subsection A.

Applicants respectfully submit that the Examiner has failed to meet the burden required to establish a *prima facie* case of obviousness because (1) SpeB pro-polypeptide and mature SpeB polypeptide function differently together than they do separately; (2) this change in function was an unexpected result; and (3) there was no reasonable expectation of success in producing *soluble* protein. Furthermore, even if a *prima facie* case of obviousness was established, it would be overcome by the evidence of failure of others to practice the claimed invention.

**1. The claimed elements function differently when co-expressed: mature SpeB polypeptide folds and becomes soluble, and SpeB pro-polypeptide enables solubility of mature SpeB polypeptide.**

To support a *prima facie* case of obviousness under this rationale, the Examiner must show that "each element merely performs the same function as it does separately." See MPEP 8th ed. rev 6, § 2143, subsection A. However, when co-expressed in the same cell, mature SpeB polypeptide ("mature SpeB") and SpeB pro-polypeptide ("pro-SpeB") do *not* perform the same function as they do separately.

When they are expressed separately, mature SpeB is insoluble, and pro-SpeB is not known to contribute to the folding of other proteins. However, when they are expressed together in the same cell, mature SpeB folds and becomes soluble, and pro-SpeB contributes to that folding and solubility. Thus, the elements of the claimed combination function differently together than they do separately, and therefore the claims are not obvious.

The Examiner correctly recognizes that the claimed elements function differently in combination than they do in isolation. The Examiner alleges that "it would have been obvious, at the time of invention, to use the plasmids of Gubba *et al.* to express both the SpeB pro-polypeptide and the mature SpeB polypeptide in the same cell, *so that the mature SpeB polypeptide would be properly folded.*" See Office Action, page 11 [emphasis added]. As the Examiner correctly acknowledges, mature SpeB functions

differently depending on whether pro-SpeB is present, i.e. is soluble rather than being insoluble.

Thus, the recited elements perform a different function together than that which they do separately. Therefore, the claims are not obvious. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

**2. The ability of pro-SpeB to promote solubility of mature SpeB through *intermolecular* interactions was an unexpected result.**

As the Examiner correctly notes, "the mature SpeB polypeptide is insoluble when expressed by itself; the pro-polypeptide portion of SpeB is necessary for proper folding and solubility." See Office Action spanning 10-11. The Examiner then alleges that "it would have been obvious, at the time of invention, to use the plasmids of Gubba *et al.* to express both the SpeB pro-polypeptide and the mature SpeB polypeptide in the same cell." See Office Action, page 11. However, this argument inappropriately equates the folding behavior of proteins that are distinct polypeptides with the behavior of fusion proteins.

It is well known in the art that the amino acid sequence of a protein dictates its three-dimensional structure. The teachings in the art indicate that protein structure is formed through *intramolecular* interactions among the amino acid residues of a polypeptide chain, and *intermolecular* interactions with the solvent and chaperones. A person of ordinary skill in the art would not expect the folding of mature SpeB to be influenced by *intermolecular* interaction with a "bystander" protein such as pro-SpeB.

Thus, the ability of pro-SpeB to promote solubility of mature SpeB through *intermolecular* interactions was an unexpected result. Therefore, the claims are not obvious. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

**3. An expectation of success in co-transduction of gene products does not indicate a reasonable expectation of success in producing *soluble* protein.**

The Examiner alleges that "One would have had a reasonable expectation of success because the vectors disclosed by Matsuka *et al.* are standard expression vectors and because expressing multiple plasmids in the same cell has been performed for many years." See Office Action, page 11.

As the Examiner correctly acknowledges, one of the requirements for a *prima facie* finding of obviousness is a reasonable expectation of success. However, a reasonable expectation of success requires more than the mere technical ability to carry out the steps of the claimed process. As provided in the MPEP:

> **2143.02 Reasonable Expectation of Success Is Required**
>
> A rationale to support a conclusion that a claim would have been obvious is that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination would have yielded nothing more than predictable results to one of ordinary skill in the art.

See MPEP 8th ed., rev. 6, § 2143.02.

Thus, the mere ability of a person skilled in the art to co-express multiple gene products in the same cell is insufficient to establish a reasonable expectation of success. Rather, to establish a reasonable expectation of success, the Examiner must also show that "the combination would have yielded nothing more than predictable results to one of ordinary skill in the art." *Id.* At the time of Applicants' invention it was thought that pro-SpeB could only contribute to the folding and stability of the mature SpeB through *intramolecular* interactions as part of the same polypeptide. That pro-SpeB would contribute to folding and solubility of mature SpeB through *intermolecular* interactions was unexpected. Thus, whether a person of ordinary skill in the art had the technical ability to perform the claimed process is irrelevant because prior to Applicants'

disclosure there was no reasonable expectation that the process would succeed in producing *soluble* protein. Absent a showing that there is a reasonable expectation of success, the claims are not *prima facie* obvious. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

**4. Failure of others to practice the claimed invention provides further indication of non-obviousness.**

A failure of others to practice the claimed invention is evidence that a claimed invention is not obvious:

> Objective evidence relevant to the issue of obviousness must be evaluated by Office personnel. Id. at 17-18, 148 USPQ at 467. Such evidence, sometimes referred to as "secondary considerations," may include evidence of commercial success, long-felt but unsolved needs, failure of others, and unexpected results.

See MPEP 8th ed., rev. 6, § 2141, subsection II.

As the Examiner correctly notes, "Matsuka *et al.* disclose that the mature SpeB polypeptide is insoluble when expressed by itself." See Office Action, page 10. Matsuka *et al.* desired mature SpeB for a research study. Having failed to produce mature SpeB directly, Matsuka *et al.* sought an alternative method. However, despite the availability of all the necessary reagents, Matsuka *et al.* did not practice the claimed invention. Instead, Matsuka *et al.* expressed SpeB zymogen and subjected it to limited proteolysis. The claimed invention would have provided mature SpeB protein of greater purity and concentration, with less effort and for lower cost, however Matsuka et al. instead obtained mature SpeB through an inferior method. See Specification, page 2, line 29 to page 3, line 15.

If the invention were obvious, Matsuka *et al.* would have used it. Instead, Matsuka *et al.* used an inferior method to obtain mature SpeB, incurring numerous disadvantages. The failure of Matsuka *et al.* to practice the claimed invention despite a clear incentive to do so provides further indication that the claimed invention is not

obvious. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

**B. Claims 1-22 are not obvious.**

The Examiner has rejected claims 1-22 as allegedly obvious over Gubba *et al.* in view of Matsuka *et al.* and in further view of Tan (Prot. Expression and purification, 21:224-234, 2001). See Office Action, page 12.

Tan teaches a polycistronic vector for protein expression in E. coli. Even if the polycistronic vector of Tan could be used to co-express pro-SpeB and mature SpeB, Tan fails to teach or suggest that mature SpeB so expressed would be soluble. Tan does not address any of the deficiencies of Gubba *et al.* in view of Matsuka *et al.*, set forth above: (1) SpeB pro-polypeptide and mature SpeB polypeptide function differently together than they do separately; (2) this change in function was an unexpected result; and (3) there was no reasonable expectation of success in producing *soluble* protein. Further, Tan does not address the failure of others to practice the claimed invention.

Thus, Tan fails to remedy any of the deficiencies of Gubba *et al.* and Matsuka *et al.* set forth above, and fails to controvert the evidence of failure of others to practice the claimed invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

**VIII. Conclusion**

Applicants respectfully request reconsideration and withdrawal of all objections and rejections. An indication of allowance of all pending claims is respectfully solicited.

In the event any issues remain, Applicants would appreciate the courtesy of a telephone call to their counsel to resolve such issues.

Respectfully submitted,

HUNTON & WILLIAMS LLP

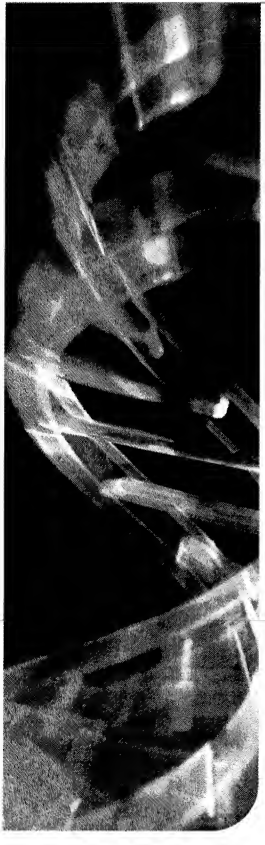
Dated: February 26, 2008 By: _____

Robert M. Schulman
Registration No. 31,196
Scott F. Yarnell
Registration No. 45,245

HUNTON & WILLIAMS
INTELLECTUAL PROPERTY DEPARTMENT
1900 K STREET, NW, SUITE 1200
WASHINGTON, D.C. 20006-1109
(202) 955-1500 (TELEPHONE)
(202) 778-2201 (FACSIMILE)

## APPENDIX A

## NOVAGEN 2004/2005 CATALOG, PAGES 116-117 AND 406



## Strain Descriptions *continued*

### Origami

Origami™ host strains are K-12 derivatives that have mutations in both the thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes, which greatly enhance disulfide bond formation in the cytoplasm. Studies have shown that expression in Origami(DE3) yielded 10-fold more active protein than in another host even though overall expression levels were similar (6). Origami hosts are compatible with ampicillin-resistant plasmids and are ideal for use with pET-32 vectors, since the thioredoxin fusion tag further enhances the formation of disulfide bonds in the cytoplasm. The *trxB* and *gor* mutations are selectable on kanamycin and tetracycline, respectively; therefore, these strains cannot be used with the plasmids carrying kanamycin or tetracycline resistance genes. To reduce the possibility of disulfide bond formation between molecules, hosts containing the *trxB/gor* mutation are recommended only for the expression of proteins that require disulfide bond formation for proper folding.

### Origami B

Origami B host strains carry the same *trxB/gor* mutations as the original Origami strains, except that they are derived from a *lacZY* mutant of BL21. Thus the Origami B strains combine the desirable characteristics of BL21, Tuner™, and Origami hosts in one strain background. The *trxB* and *gor* mutations are selectable on kanamycin and tetracycline, respectively; therefore, these strains cannot be used with the plasmids carrying kanamycin or tetracycline resistance genes.

### Rosetta and Rosetta 2

Rosetta™ and Rosetta 2 host strains are BL21 derivatives designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli*. The original Rosetta strains supply tRNAs for the codons AUA, AGG, AGA, CUA, CCC, and GGA on a compatible chloramphenicol-resistant plasmid. The Rosetta 2 strains supply a seventh rare codon (CGG) in addition to the six found in the original Rosetta strains.

By supplying rare codons, the Rosetta strains provide for "universal" translation where translation would otherwise be limited by the codon usage of *E. coli*. The tRNA genes are driven by their native promoters. In the pLysS and pLacI derivatives of these strains, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively.

### RosettaBlue

RosettaBlue™ host strains are NovaBlue derivatives that combine high transformation efficiency and *recA endA lacI*$^{q}$ mutations with enhanced expression of eukaryotic proteins that contain codons rarely used in *E. coli*. These strains supply tRNAs for AGG, AGA, AUA, CUA, CCC, and GGA on a compatible chloramphenicol-resistant plasmid. The tRNA genes are driven by their native promoters. In RosettaBlue(DE3)pLysS and RosettaBlue(DE3)pLacI, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively. Blue/white screening is not possible with RosettaBlue(DE3) strains due to the presence of the *lacZ* α-peptide coding sequence in the λDE3 prophage.

### Rosetta-gami

Rosetta-gami™ host strains are Origami derivatives that combine the enhanced disulfide bond formation resulting from *trxB/gor* mutations with enhanced expression of eukaryotic proteins that contain codons rarely used in *E. coli*. These strains supply tRNAs for AGG, AGA, AUA, CUA, CCC, and GGA on a compatible chloramphenicol-resistant plasmid. The tRNA genes are driven by their native promoters. In Rosetta-gami(DE3)pLysS and Rosetta-gami(DE3)pLacI, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively. The *trxB* and *gor* mutations are selectable on kanamycin and tetracycline, respectively; therefore, these strains cannot be used with the plasmids carrying kanamycin or tetracycline resistance genes.

### Rosetta-gami B

Rosetta-gami B strains combine the key features of BL21 (and its Tuner™ derivative), Origami, and Rosetta to enhance both the expression of eukaryotic proteins and the formation of target protein disulfide bonds in the bacterial cytoplasm. These strains are compatible with ampicillin- or spectinomycin-resistant vectors.

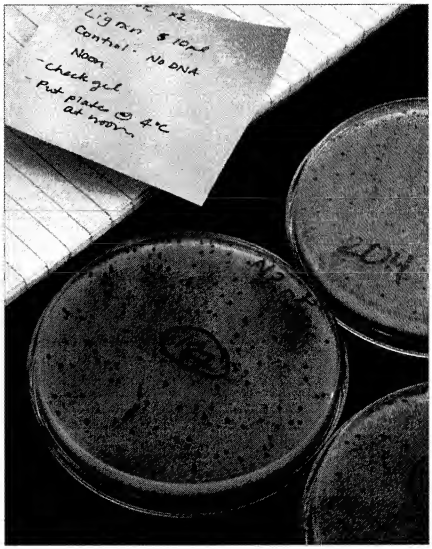
*continued on the next page*

## Strain Descriptions *continued*

Tuner

Tuner™ strains are *lacZY* deletion mutants of BL21 and enable adjustable levels of protein expression throughout all cells in a culture. The *lac* permease (*lacY*) mutation allows uniform entry of IPTG into all cells in the population, which produces a concentration-dependent, homogeneous level of induction. By adjusting the concentration of IPTG, expression can be regulated from very low levels up to the robust, fully induced levels commonly associated with pET vectors. Lower-level expression may enhance the solubility and activity of difficult target proteins.

1. Wood, W. B. (1966) *J. Mol. Biol.* 16, 118–133.
2. Leahy, D. J., Hendrickson, W. A., Aukhil, I., and Erickson, H. P. (1992) *Science* 258, 987–991.
3. Phillips, T. A., Van Bogelen, R. A., and Neidhardt, F. C. (1984) *J. Bacteriol.* 159, 283–287.
4. A. Roca (University of Wisconsin-Madison), personal communication.
5. Studier, F. W. (1991) *J. Mol. Biol.* 219, 37–44.
6. Prinz, W. A., Aslund, F., Holmgren, A., and Beckwith, J. (1997) *J. Biol. Chem.* 272, 15661–15667.



## Strain Descriptions *continued*

### Features and Applications of Novagen Competent Cell Strains

| Strain | Strain background | Protein expression: pET[1] | Protein expression: pCDF | Protein expression: pRSF | Protein expression: pACYDuet™ | Protein expression: pETBlue™ | Protein expression: pTriEx™ | Protein expression: non-T7[2] | *recA*[3, 4] | *endA*[3, 5] | Blue/white screening[6] | *lacI*[q][7] | F′ episome[8] | *ompT*[3, 9] | *lon*[3, 10] | *trxB*[3, 11] | *gor*[3, 12] | *lacY*[1, 13] | Rare codon tRNAs[14] | pLysS[15] | pLacI[16] | *mcrA*[17] | *dcm*[3, 18] | Available as Singles™ | Available as Quartets™ | Available as HTFit™ | Chloramphenicol resistance | Kanamycin resistance | Tetracycline resistance | Chemically Competent | Electrocompetent |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B834(DE3) | B | ✓ | ✓ | ✓ | ✓ | | | | | | | | | ✓ | ✓ | | | | | | ✓ | ✓ | | | | | | | ✓ | | |
| B834(DE3)pLysS | B | ✓ | ✓ | ✓ | | | | | | | | | | ✓ | ✓ | | | | | ✓ | ✓ | ✓ | | | | | | | ✓ | | |
| BL21 | B | | | | | | | | ✓ | | | | | ✓ | ✓ | | | | | | | | ✓ | | ✓ | | | | ✓ | | |
| BL21(DE3) | B | ✓ | ✓ | ✓ | ✓ | | | | | | | | | ✓ | ✓ | | | | | | | | ✓ | ✓ | ✓ | ✓ | | | ✓ | | |
| BL21(DE3)pLysS | B | ✓ | ✓ | ✓ | | | | | | | | | | ✓ | ✓ | | | | | ✓ | | | ✓ | ✓ | ✓ | | ✓ | | ✓ | | |
| BLR | B | | | | | | | | ✓ | ✓ | | | | ✓ | ✓ | | | | | | | | ✓ | | ✓ | | | | ✓ | ✓ | |
| BLR(DE3) | B | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | | | | ✓ | ✓ | | | | | | | | ✓ | | | | | | ✓ | ✓ | |
| BLR(DE3)pLysS | B | ✓ | ✓ | ✓ | | | | | | ✓ | | | | ✓ | ✓ | | | | | ✓ | | | ✓ | | | | ✓ | | ✓ | ✓ | |
| HMS174 | K-12 | | | | | | | | ✓ | ✓ | | | | | | | | | | | | | | | | | | | Rif | ✓ | |
| HMS174(DE3) | K-12 | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | | | | | | | | | | | | | | | | | | | Rif | ✓ | |
| HMS174(DE3)pLysS | K-12 | ✓ | ✓ | ✓ | | | | | | ✓ | | | | | | | | | | ✓ | | | | | | | ✓ | | Rif | ✓ | |
| NovaBlue | K-12 | | | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | | | | | | ✓ | | ✓ | | | ✓ | ✓ | |
| NovaBlue(DE3) | K-12 | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | ✓ | | ✓ | ✓ | | | | ✓ | | | | | | | | | | | ✓ | ✓ | |
| NovaF′ | K-12 | | | | | | | | ✓ | ✓ | ✓ | | | | | | | | | | | | | | | | | | ✓ | | |
| NovaXG | K-12 | | | | | | | | ✓ | ✓ | ✓ | ✓ | | | | | | | | | | | | | | | | | | | ✓ |
| NovaXGF′ | K-12 | | | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | | | | | | | | | | | ✓ | | ✓ |
| Origami™ | K-12 | | | | | | | | ✓ | | | | ✓ | ✓ | | ✓ | ✓ | | | | | | | | | | | | ✓ | ✓ | |
| Origami(DE3) | K-12 | ✓[3] | ✓ | | ✓ | | | | | | | | ✓ | ✓ | | ✓ | ✓ | | | | | | | ✓ | | | | | ✓ | ✓ | |
| Origami(DE3)pLysS | K-12 | ✓[3] | ✓ | | | | | | | | | | ✓ | ✓ | | ✓ | ✓ | | | ✓ | | | | ✓ | | | | ✓ | ✓ | ✓ | |
| Origami(DE3)pLacI | K-12 | | | | | | ✓ | ✓ | | | | | ✓ | ✓ | | ✓ | ✓ | | | | ✓ | | | | | | | ✓ | ✓ | ✓ | |
| Origami B | B | | | | | | | | ✓ | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | | ✓ | | | | ✓ | ✓ | |
| Origami B(DE3) | B | ✓[3] | ✓ | | ✓ | | | | | | | | | ✓ | ✓ | ✓ | ✓ | | | | | | ✓ | | ✓ | | | | ✓ | ✓ | |
| Origami B(DE3)pLysS | B | ✓[3] | ✓ | | | | | | | | | | | ✓ | ✓ | ✓ | ✓ | | | ✓ | | | ✓ | | ✓ | | ✓ | ✓ | ✓ | ✓ | |
| Origami B(DE3)pLacI | B | | | | | ✓ | ✓ | | | | | | | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | ✓ | | ✓ | | ✓ | ✓ | ✓ | ✓ | |

1. Plasmids in which protein expression is controlled by a T7 promoter require host strains that carry the gene for T7 RNA polymerase. T7-based expression can also be performed with non-λDE3 lysogens by infection with λCE6.
2. Compatible with ampicillin-resistant plasmids. Not recommended for plasmids carrying the kanamycin resistance gene.
3. Suitable for expression vectors having an *E. coli* promoter (e.g., *tac*, *trc*, *trc*Ho, T5), or for T7-based expression by infection with λCE6. Expression vector should not have the same antibiotic resistance marker present in the host.
4. Lacks homologous recombination. Useful for stabilizing plasmids carrying tandem repeats, and for prevention of plasmid multimerization.
5. Lacks endonuclease for improved quality of plasmid preps.
6. Provides *lacZ*ΔM15 for α-complementation of ω fragment for β-galactosidase activity.
7. Carries *lacI*[q] for overexpression of *lac* repressor protein, which suppresses basal expression from promoters containing appropriate *lac* operator sequences.
8. F′ enables rescue of single-stranded plasmid DNA by M13 helper phage infection when using plasmids having an f1 origin of replication.
9. Lacks *ompT* membrane protease, which can cleave some recombinant proteins during purification.
10. Deficient in cytoplasmic *lon* protease.
11. Lacks thioredoxin reductase, thereby facilitating formation of disulfide bonds in the cytoplasm.
12. Lacks glutathione reductase, which, when combined with *trxB* mutation, greatly facilitates formation of disulfide bonds in the cytoplasm.
13. Lacks *lac* permease, which provides for homogeneous uptake of IPTG into all cells in the population, facilitating concentration-dependent induction of protein expression.
14. Provides tRNAs for codons that rarely occur in *E. coli*, which increases the expression level of proteins otherwise limited by codon usage.
15. Provides T7 lysozyme to reduce basal expression of target genes and therefore stabilize plasmids that express proteins toxic to *E. coli*. Even greater stringency is provided by pLysE hosts; these are available separately as glycerol stocks.
16. Overproduces *lac* repressor from a compatible plasmid, to suppress basal transcription of target genes controlled by appropriately placed *lac* operators. Designed for use with pETBlue and pTriEx constructs
17. Methionine auxotroph facilitates metabolic labeling with methionine analogs.
18. Lacks methylation of internal cytosine residues in the sequences CCAGG and CCTGG at the C[5] position.

# Strain Descriptions *continued*

## Features and Applications of Novagen Competent Cell Strains *continued*

| Strain | Strain background | Protein expression: pET[1] | Protein expression: pCDF[1] | Protein expression: pRSF[1] | Protein expression: pACYDuet™[1] | Protein expression: pETBlue™[1] | Protein expression: pTriEx™[1] | Protein expression: non-T7[2] | *recA*[3,4] | *endA*[4,5] | Blue/white screening[6] | *lacI*[7,8] | F′ episome[9] | *ompT*[10,11] | *lon*[10] | *trxB*[10,11] | *gor*[10,12] | *lacY*[13] | Rare codon tRNAs[14] | pLysS[15] | pLacI[16] | *mcrA*[17] | *dcm*[17,18] | Available as Singles™ | Available as Duartes™ | Available as HTMs™ | Chloramphenicol resistance | Kanamycin resistance | Tetracycline resistance | Chemically Competent | Electrocompetent |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rosetta™ 2 | B | | | | | | | ✓ | | | | | | ✓ | ✓ | | | | ✓ | | | | ✓ | | | | | ✓ | | ✓ | |
| Rosetta 2(DE3) | B | ✓ | ✓ | ✓ | ✓ | | | | | | | | | ✓ | ✓ | | | | ✓ | | | | ✓ | ✓ | | | | ✓ | | ✓ | |
| Rosetta 2(DE3)pLysS | B | ✓ | ✓ | ✓ | | | | | | | | | | ✓ | ✓ | | | | ✓ | ✓ | | | ✓ | ✓ | | | | ✓ | | ✓ | |
| Rosetta 2(DE3)pLacI | B | | | | | ✓ | ✓ | | | | | | | ✓ | ✓ | | | | ✓ | | ✓ | | ✓ | | | | | ✓ | | ✓ | |
| Rosetta | B | | | | | | | ✓ | | | | | | ✓ | ✓ | | | | ✓ | | | | ✓ | | ✓ | | | ✓ | | ✓ | |
| Rosetta(DE3) | B | ✓ | ✓ | ✓ | ✓ | | | | | | | | | ✓ | ✓ | | | | ✓ | | | | ✓ | | ✓ | | | ✓ | | ✓ | |
| Rosetta(DE3)pLysS | B | ✓ | ✓ | ✓ | | | | | | | | | | ✓ | ✓ | | | | ✓ | ✓ | | | ✓ | | ✓ | | | ✓ | | ✓ | |
| Rosetta(DE3)pLacI | B | | | | | ✓ | ✓ | | | | | | | ✓ | ✓ | | | | ✓ | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | |
| RosettaBlue™ | K-12 | | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | ✓ | | | | | | | | | ✓ | ✓ | ✓ | |
| RosettaBlue(DE3) | K-12 | ✓ | ✓ | ✓ | ✓ | | | | ✓ | ✓ | | ✓ | ✓ | | | | | ✓ | ✓ | | | | | | | | | ✓ | ✓ | ✓ | |
| RosettaBlue(DE3)pLysS | K-12 | ✓ | ✓ | ✓ | | | | | ✓ | ✓ | | ✓ | ✓ | | | | | ✓ | ✓ | ✓ | | | | | | | | ✓ | ✓ | ✓ | |
| RosettaBlue(DE3)pLacI | K-12 | | | | | ✓ | ✓ | | ✓ | ✓ | | ✓ | ✓ | | | | | ✓ | ✓ | | ✓ | | | | | | | ✓ | ✓ | ✓ | |
| Rosetta-gami™ | K-12 | | | | | | | ✓ | | | | ✓ | ✓ | | | ✓ | ✓ | | ✓ | | | | | | | | | ✓ | ✓ | ✓ | ✓ |
| Rosetta-gami(DE3) | K-12 | ✓[2] | ✓ | | | ✓ | | | | | | ✓ | ✓ | | | ✓ | ✓ | | ✓ | | | | | | | | | ✓ | ✓ | ✓ | ✓ |
| Rosetta-gami(DE3)pLysS | K-12 | ✓[2] | ✓ | | | | | | | | | ✓ | ✓ | | | ✓ | ✓ | | ✓ | ✓ | | | | | | | | ✓ | ✓ | ✓ | ✓ |
| Rosetta-gami(DE3)pLacI | K-12 | | | | | ✓ | ✓ | | | | | ✓ | ✓ | | | ✓ | ✓ | | ✓ | | ✓ | | | | | | | ✓ | ✓ | ✓ | ✓ |
| Rosetta-gami B | B | | | | | | | ✓ | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | ✓ | | | ✓ | ✓ | ✓ | ✓ |
| Rosetta-gami B(DE3) | B | ✓[2] | ✓ | | | ✓ | | | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | ✓ | | | ✓ | ✓ | ✓ | ✓ |
| Rosetta-gami B(DE3)pLysS | B | ✓[2] | ✓ | | | | | | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | ✓ | | ✓ | | | ✓ | ✓ | ✓ | ✓ |
| Rosetta-gami B(DE3)pLacI | B | | | | | | | ✓ | ✓ | ✓ | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | | ✓ | | | ✓ | ✓ | ✓ | ✓ |
| Tuner™ | B | | | | | | | ✓ | | | | | | ✓ | ✓ | | | | ✓ | | | | ✓ | | | | | | | ✓ | |
| Tuner(DE3) | B | ✓ | ✓ | ✓ | ✓ | | | | | | | | | ✓ | ✓ | | | | ✓ | | | | ✓ | | | | | | | ✓ | |
| Tuner(DE3)pLysS | B | ✓ | ✓ | ✓ | | | | | | | | | | ✓ | ✓ | | | | ✓ | ✓ | | | ✓ | | | | | ✓ | | ✓ | |
| Tuner(DE3)pLacI | B | | | | | ✓ | ✓ | | | | | | | ✓ | ✓ | | | | ✓ | | ✓ | | ✓ | | | | | ✓ | | ✓ | |

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3. Suitable for expression vectors having an *E. coli* promoter (e.g., *tac, trc*, *trc*Ttis, T5), or for T7-based expression by infection with λCE6. Expression vector should not have the same antibiotic resistance marker present in the host.
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6. Provides *lacZ*ΔM15 for α-complementation of ω fragment for β-galactosidase activity.
7. Carries *lacI*$^{q}$ for overexpression of *lac* repressor protein, which suppresses basal expression from promoters containing appropriate *lac* operator sequences.
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12. Lacks glutathione reductase, which, when combined with *trxB* mutation, greatly facilitates formation of disulfide bonds in the cytoplasm.
13. Lacks *lac* permease, which provides for homogeneous uptake of IPTG into all cells in the population, facilitating concentration-dependent induction of protein expression.
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18. Lacks methylation of internal cytosine residues in the sequences CCAGG and CCTGG at the C$^{5}$ position.



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